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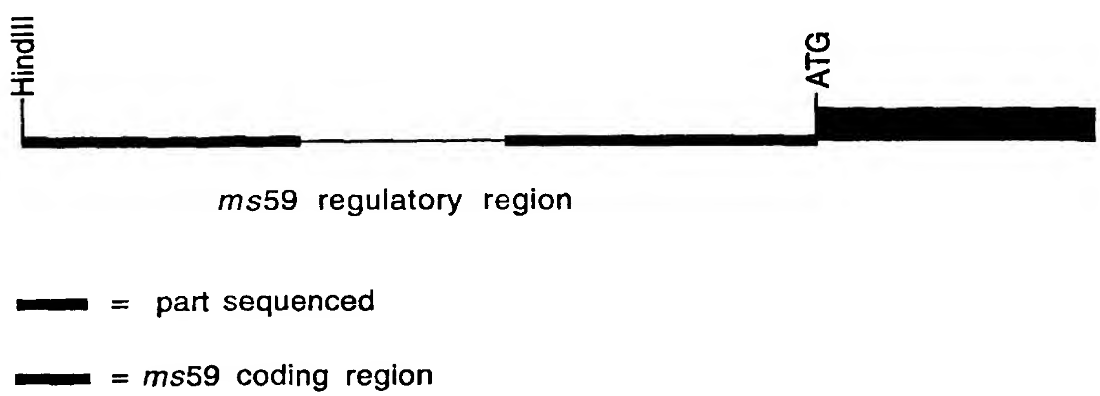

(57) Abstract

This invention describes pathogen-inducible promoters which normally drive expression of plant hexose oxidases, especially those which can be isolated from *Helianthus annuus* and *Lactuca sativa*, more specifically those promoters which naturally are the regulatory regions driving expression of the hexose oxidase MS59 and WL64, respectively. Also claimed are chimeric constructs where these pathogen-inducible promoters drive expression of antipathogenic proteins or of proteins which can elicit a hypersensitive response.

Bao, et al.
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PATHOGEN-INDUCIBLE PROMOTER

FIELD OF THE INVENTION

This invention is related to the field of pathogen-inducible promoters, and chimeric DNA sequences comprising said promoters, especially in the area of plant biotechnology.

BACKGROUND ART

Inducible promoters include any promoter capable of increasing the amount of gene product produced by a given gene, in response to exposure to an inducer. In the absence of an inducer the DNA sequence will not be transcribed. Typically, the factor that binds specifically to an inducible promoter to activate transcription is present in an inactive form which is then directly or indirectly converted to the active form by the inducer. The inducer may be a chemical agent such as a protein, metabolite (sugar, alcohol, etc.), a growth regulator, herbicide, or a phenolic compound or a physiological stress imposed directly by heat, salt, wounding, toxic elements etc., or indirectly through the action of a pathogen or disease agent such as a virus. A plant cell containing an inducible promoter may be exposed to an inducer by externally applying the inducer to the cell such as by spraying, watering, heating, or similar methods. Inducible promoters are known to those familiar with the art and several exist that are be used to drive expression of genes of interest. Examples of inducible promoters include the inducible 70 kD heat shock promoter of *Drosophila melanogaster* (Freeling, M. *et al.*, Ann. Rev. Genet. 19, 297-323) and the alcohol dehydrogenase promoter which is induced by ethanol (Nagao, R.T. *et al.*, in: Miflin, B.J. (ed.) Oxford Surveys of Plant Molecular and Cell Biology, Vol. 3., pp. 384-438, Oxford Univ. Press, 1986). Examples for promoters that are inducible by a simple chemical are the promoters described in WO 90/08826, WO 93/21334, WO 93/031294 and WO 96/37609.

An important subclass of inducible promoters are the promoters which are induced in plants upon pathogen infection. As examples of a pathogen-inducible promoter the PRP1 promoter (also named gst1 promoter) obtainable from potato (Martini N. *et al.* (1993), Mol. Gen.

Genet. 263, 179-186), the *Fis1* promoter (WO 96/34949), the *Bet v 1* promoter (Swoboda, I., *et al.*, Plant, Cell and Env. 18, 865-874, 1995), the *Vst1* promoter (Fischer, R., Dissertation, Univ. of Hohenheim, 1994; Schubert, R., *et al.* Plant Mol. Biol. 34, 417-426, 1997), the sesquiterpene cyclase promoter (Yin, S., *et al.*, Plant Physiol. 115, 437-451, 1997) and the *gstA1* promoter (Mauch, F. and Dudler, R., Plant Physiol. 102, 1193-1201, 1993) may be mentioned. A drawback of some of these promoters is that they are also active constitutively or that they do not react to certain types of pathogens. Furthermore, it would be advantageous to have promoters that regulate expression very soon after pathogen infection, *i.e.* with as short as possible induction times.

Thus, there is still need for promoters that are pathogen-inducible which overcome the disadavantages of the prior art.

SUMMARY OF THE INVENTION

We now have found DNA fragments which are the upstream regulatory regions for plant genes coding for hexose oxidase, capable of promoting pathogen-inducible transcription of an associated DNA sequence when re-introduced into a plant. Preferably such a fragment is obtainable *from Helianthus annuus*. Said DNA fragment specifically is the upstream regulatory region of the gene coding for hexose oxidase, denoted as MS59, more specifically characterized in that it comprises the nucleotide sequence from 1 to 1889 depicted in SEQ ID NO: 15.

Also part of the invention is a DNA fragment obtainable from *Lactuca sativa*, capable of promoting pathogen-inducible transcription of an associated DNA sequence when re-introduced into a plant, specifically the DNA fragment that it is the upstream regulatory region of the gene coding for hexose oxidase, denoted as WL64 (SEQ ID NO: 18).

Also included in the invention is a portion or variant of a DNA fragment according to any described above, capable of promoting pathogen-inducible transcription of an associated DNA sequence when re-introduced into a plant.

Embodiments of the invention are chimeric DNA sequences comprising in the direction of transcription a DNA fragment according to any one of the DNA fragments described above and a DNA sequence to be expressed under the transcriptional control thereof and which is not naturally under transcriptional control of said DNA fragment. A preferred embodiment is such a chimeric DNA sequence wherein the DNA sequence to be expressed causes the production of an antipathogenic protein, which is preferably selected from the group consisting of chitinase, glucanase, osmotin, magainins, lectins, saccharide oxidase, oxalate oxidase, toxins from *Bacillus thuringiensis*, antifungal proteins isolated from *Mirabilis jalapa, Amaranthus, Raphanus, Brassica, Sinapis, Arabidopsis, Dahlia, Cnicus, Lathyrus, Clitoria, Allium* seeds, *Aralia* and *Impatiens* and albumin-type proteins, such as thionine, napin, barley trypsin inhibitor, cereal gliadin and wheat-alpha-amylase.
Another embodiment of the chimeric DNA sequences of the invention is a chimeric DNA sequence wherein the DNA sequence to be expressed causes the production of a protein that can induce a hypersensitive response, preferably selected from the group consisting of CF and Pto proteins from tomato, avr proteins from *Cladosporium fulvum* and elicitor proteins from *Pseudomonas* or *Xanthomonas*.

Further part of the invention are replicons comprising above mentioned chimeric DNA sequences preferably having at least one recognition site for a restriction endonuclease for insertion of a DNA sequence to be expressed under the control of said DNA fragment. Also included in the invention are microorganisms containing such a replicon, plant cells having incorporated into their genome a chimeric DNA sequence according to those described above, and plants essentially consisting of said cells. Such a plant is preferably a dicotyledonous plant. Also part of said plants selected from seeds, flowers, tubers, roots, leaves, fruits, pollen and wood, form part of the invention.

Yet another embodiment of the invention is the use of a DNA fragment as described above for identifying homologues capable of promoting pathogen-induced transcription in a plant.

Further use of a chimeric DNA sequence according to the invention for transforming plants and use of a portion or variant of

the DNA fragments according to the invention for making hybrid regulatory DNA sequences is part of the invention.

Another object of the invention is the use of a chimeric DNA sequence as described above for conferring pathogen resistance to a plant.

DESCRIPTION OF THE FIGURES

Fig. 1. Schematic drawing of (genomic) ms59 gene and promoter region.

DETAILED DESCRIPTION OF THE INVENTION

The main aspect of the invention are regulatory sequences naturally occurring in plants and driving the expression of genes coding for hexose oxidase. Specifically the regions occurring in *Helianthus annuus* and/or *Lactuca sativa*, especially in the 5' (upstream) region of the MS59-gene (SEQ ID NO:15) or the WL64-gene (SEQ ID NO: 17), respectively. These genes encode a hexose oxidase which is found to be toxic to (fungal) pathogens, and they are disclosed in WO 98/13478, which is hereby inclosed for reference. It has been found that upon pathogen infection these genes are highly expressed, indicating pathogen inducibility. Pathogen inducible promoters are of great value in biotechnological resistance engineering.

Although the invention is exemplified especially with respect to the promoter driving the expression of the hexose oxidase in sunflower, denoted as 'ms59', it is believed that all promoters driving genes homologous to this ms59 will have properties that are more or less identical to the promoter of ms59, i.e. expression activity which is induced by pathogen infection, preferably by fungal infection. It is commonly known that promoters of homologous genes share similar properties, especially related to induction by pathogens. Examples of these are the promoters driving expression of the pathogenesis related protein osmotin, of which in potato (Zhu *et al.*, Plant Physiol. 108, 929-937,1995), in tobacco (Liu *et al.*,Plant Mol. Biol. 29, 1015-1026, 1995) and in tomato (Ruiz-Medrano *et al.*, Plant Mol. Biol. 20, 1199-1202, 1992) the inducibility by pathogens has been established; and promoters driving the expression of genes of the PR-10 group, of which the pSTH-2 promoter in potato (Matton, D.P. and Brisson, N, Mol. Plant-Microbe Interac. 2, 325-331, 1989), the AoPR-1 promoter in *Asparagus officinalis* (Warner, *et al.*, The Plant J. 3, 191-201, 1993) and the Bet v 1 promoter in *Betula verrucosa* (Swoboda *et al.*, Plant, Cell, Environm. 18, 865-874, 1995) have been shown to be pathogen inducible.

In this desription the terms 'regulatory sequence' and 'promoter' are used interchangeably.

The present invention provides amongst others chimeric DNA sequences which comprise the regulatory sequences according to the invention. The expression chimeric DNA sequence shall mean to comprise any DNA sequence which comprises DNA sequences not naturally found in nature. For instance, chimeric DNA shall mean to comprise DNA non-comprising the regulatory region which is pathogen-inducible in a non-natural location of the plant genome, notwithstanding the fact that said plant genome normally contains a copy of the said regulatory region in its natural chromosomal location. Similarly, the said regulatory region may be incorporated in the plant genome wherein it is not naturally found, or in a replicon or vector where it is not naturally found, such as a bacterial plasmid or a viral vector. Chimeric DNA shall not be limited to DNA molecules which are replicable in a host, but shall also mean to comprise DNA capable of being ligated into a replicon, for instance by virtue of specific adaptor sequences, physically linked to the regulatory region according to the invention. The regulatory region may or may not be linked to its natural downstream open reading frame.

The open reading frame of the gene which expression is driven by the pathogen-inducible regulatory regions of the invention may be derived from a genomic library. In this latter it may contain one or more introns separating the exons making up the open reading frame that encodes a protein according to the invention. The open reading frame may also be encoded by one uninterrupted exon, or by a cDNA to the mRNA encoding a protein according to the invention. Chimeric DNA sequences according to the invention also comprise those in which one or more introns have been artificially removed or added. Each of these variants is embraced by the present invention.

In order to be capable of being expressed in a host cell a regulatory region according to the invention will usually be provided with a transcriptional initiation region which may be suitably derived from any gene capable of being expressed in the host cell of choice, as well as a translational initiation region for ribosome recognition and attachment. In eukaryotic cells, an expression cassette usually comprises in addition a transcriptional termination region located downstream of said open reading frame, allowing transcription to

terminate and polyadenylation of the primary transcript to occur. In addition, the codon usage may be adapted to accepted codon usage of the host of choice. Further, often a signal sequence may be encoded, which is responsible for the targeting of the gene expression product to subcellular compartments. The principles governing the expression of a chimeric DNA construct in a chosen host cell are commonly understood by those of ordinary skill in the art and the construction of expressible chimeric DNA constructs is now routine for any sort of host cell, be it prokaryotic or eukaryotic.

In order for the chimeric DNA sequence to be maintained in a host cell it will usually be provided in the form of a replicon comprising said chimeric DNA sequence according to the invention linked to DNA which is recognised and replicated by the chosen host cell. Accordingly, the selection of the replicon is determined largely by the host cell of choice. Such principles as govern the selection of suitable replicons for a particular chosen host are well within the realm of the ordinary skilled person in the art.

A special type of replicon is one capable of transferring itself, or a part thereof, to another host cell, such as a plant cell, thereby co-transferring the open reading frame according to the invention to said plant cell. Replicons with such capability are herein referred to as vectors. An example of such vector is a Ti-plasmid vector which, when present in a suitable host, such as *Agrobacterium tumefaciens*, is capable of transferring part of itself, the so-called T-region, to a plant cell. Different types of Ti-plasmid vectors (*vide:* EP 0 116 718 B1) are now routinely being used to transfer chimeric DNA sequences into plant cells, or protoplasts, from which new plants may be generated which stably incorporate said chimeric DNA in their genomes. A particularly preferred form of Ti-plasmid vectors are the so-called binary vectors as claimed in (EP 0 120 516 B1 and US 4,940,838). Other suitable vectors, which may be used to introduce DNA according to the invention into a plant host, may be selected from the viral vectors, *e.g.* non-integrative plant viral vectors, such as derivable from the double stranded plant viruses (*e.g.* CaMV) and single stranded viruses, gemini viruses and the like. The use of such vectors may be advantageous, particularly when it is difficult to stably transform the plant host. Such may be the case with woody species, especially trees and vines.

The expression "host cells incorporating a chimeric DNA sequence according to the invention in their genome" shall mean to comprise cells, as well as multicellular organisms comprising such cells, or essentially consisting of such cells, which stably incorporate said chimeric DNA into their genome thereby maintaining the chimeric DNA, and preferably transmitting a copy of such chimeric DNA to progeny cells, be it through mitosis or meiosis. According to a preferred embodiment of the invention plants are provided, which essentially consist of cells which incorporate one or more copies of said chimeric DNA into their genome, and which are capable of transmitting a copy or copies to their progeny, preferably in a Mendelian fashion. By virtue of the transcription and translation of the chimeric DNA according to the invention in some or all of the plant's cells, those cells that comprise said regulatory region will respond to pathogen attack and thus produce the protein encoded by the open reading frame which is under control of the regulatory region. In specific embodiments of the invention this protein will be an antipathogenic protein which is capable of conferring resistance to pathogen infections.

As is well known to those of skill in the art, regulatory regions of plant genes consist of disctinct subregions with interesting properties in terms of gene expression. Examples of subregions as meant here, are enhancers but also silencers of transcription. These elements may work in a general (constitutive) way, or in a tissue-specific manner. Deletions may be made in the regulatory DNA sequences according to the invention, and the subfragments may be tested for expression patterns of the associated DNA. Various subfragments so obtained, or even combinations thereof, may be useful in methods of engineering pathogen resistance, or other applications involving the expression of heterologous DNA in plants. The use of DNA sequences according to the invention to identify functional subregions, and the subsequent use thereof to promote or suppress gene expression in plants is also encompassed by the present invention.

As regards the necessity of a transcriptional terminator region, it is generally believed that such a region enhances the reliability as well as the efficiency of transcription in plant cells. Use thereof is therefore strongly preferred in the context of the present invention.

Examples of proteins that may be used in combination with the ICS regulatory region according to the invention include, but are not limited to, ß-1,3-glucanases and chitinases which are obtainable from barley (Swegle M. *et al.*, Plant Mol. Biol. 12, 403-412, 1989; Balance G.M. *et al.*, Can. J. Plant Sci. 56, 459-466, 1976 ; Hoj P.B. *et al.*, FEBS Lett. 230, 67-71, 1988; Hoj P.B. *et al.*, Plant Mol. Biol. 13, 31-42, 1989), bean (Boller T. *et al.*, Planta 157, 22-31, 1983; Broglie K.E. *et al.*, Proc. Natl. Acad. Sci. USA 83, 6820-6824, 1986; Vögeli U. *et al.*, Planta 174, 364-372, 1988); Mauch F. & Staehelin L.A., Plant Cell 1, 447-457, 1989); cucumber (Metraux J.P. & Boller T., Physiol. Mol. Plant Pathol. 28, 161-169, 1986); leek (Spanu P. *et al.*, Planta 177, 447-455, 1989); maize (Nasser W. *et al.*, Plant Mol. Biol. 11, 529-538, 1988), oat (Fink W. *et al.*, Plant Physiol. 88, 270-275, 1988), pea (Mauch F. *et al.*, Plant Physiol. 76, 607-611, 1984; Mauch F. *et al.*, Plant Physiol. 87, 325-333, 1988), poplar (Parsons, T.J. *et al.*, Proc. Natl. Acad. Sci. USA 86, 7895-7899, 1989), potato (Gaynor J.J., Nucl. Acids Res. 16, 5210, 1988; Kombrink E. *et al.*, Proc. Natl. Acad. Sci. USA 85, 782-786, 1988; Laflamme D. and Roxby R., Plant Mol. Biol. 13, 249-250, 1989), tobacco (e.g. Legrand M. *et al.*, Proc. Natl. Acad. Sci. USA 84, 6750-6754, 1987; Shinshi H. *et al.* Proc. Natl. Acad. Sci. USA 84, 89-93, 1987), tomato (Joosten M.H.A. & De Wit P.J.G.M., Plant Physiol. 89, 945-951, 1989), wheat (Molano J. *et al.*, J. Biol. Chem. 254, 4901-4907, 1979), magainins, lectins, toxins isolated from *Bacillus thuringiensis*, antifungal proteins isolated from *Mirabilis jalapa* (EP 0 576 483) and *Amaranthus* (EP 0 593 501 and US 5,514,779), albumin-type proteins (such as thionine, napin, barley trypsin inhibitor, cereal gliadin and wheat-alpha-amylase, EP 0 602 098), proteins isolated from *Raphanus, Brassica, Sinapis, Arabidopsis, Dahlia, Cnicus, Lathyrus* and *Clitoria* (EP 0 603 216), proteins isolated from *Capsicum, Briza, Delphinium, Catapodium, Baptisia* and *Microsensis* (PCT/GB93/02179), oxalate oxidase (EP 0 636 181 and EP 0 673 416), saccharide oxidase (PCT/EP 97/04923), antimicrobial proteins isolated from *Allium* seeds (PCT/GB94/01636), proteins from *Aralia* and *Impatiens* (PCT/GB95/00509), proteins from *Heuchera* and *Aesculus* (PCT/GB94/02766), mutant peptides to the above mentioned proteins (PCT/GB96/03065 and PCT/GB96/03068) and the like.

Another use of the inducible promoter is to drive proteins which play a role in the gene-for-gene resistance interaction (e.g. as described in WO 91/15585). Such proteins are, for example, plant proteins such as disclosed in Karrer, E.E. *et al.* (Plant Mol. Biol. 36, 681-690, 1998), activated ndr1, activated eds1 and activated Xa21, Cf-proteins, BS3 protein and Pto proteins from tomato, Rpm1 and Rps2 proteins from *Arabidopsis thaliana*, the N-gene from tobacco, the avr-elicitor proteins from *Cladosporium fulvum*, avrBs3 from Xanthomonas, harpins from *Erwinia* and the *avrPto* protein from *Pseudomonas*,

The actual applicability of the invention is not limited to certain plant species. Any plant species that is subject to some form of pathogen attack, may be transformed with chimeric DNA sequences according to the invention, allowing the regulatory region to be induced by pathogen infection thereby triggering production of antipathogenic proteins to be produced in some or all of the plant's cells.

Although some of the embodiments of the invention may not be practicable at present, *e.g.* because some plant species are as yet recalcitrant to genetic transformation, the practicing of the invention in such plant species is merely a matter of time and not a matter of principle, because the amenability to genetic transformation as such is of no relevance to the underlying embodiment of the invention.

Transformation of plant species is now routine for an impressive number of plant species, including both the *Dicotyledoneae* as well as the *Monocotyledoneae*. In principle any transformation method may be used to introduce chimeric DNA according to the invention into a suitable ancestor cell, as long as the cells are capable of being regenerated into whole plants. Methods may suitably be selected from the calcium/polyethylene glycol method for protoplasts (Krens, F.A. *et al.*, Nature 296, 72-74, 1982; Negrutiu I. *et al*,, Plant Mol. Biol. 8, 363-373, 1987), electroporation of protoplasts (Shillito R.D. *et al.*, Bio/Technol. 3, 1099-1102, 1985), microinjection into plant material (Crossway A. *et al.*, Mol. Gen. Genet. 202, 179-185, 1986), DNA (or RNA-coated) particle bombardment of various plant material (Klein T.M. *et al.*, Nature 327, 70, 1987), infection with (non-integrative) viruses and the like. A preferred method according to the invention

comprises *Agrobacterium*-mediated DNA transfer. Especially preferred is the use of the so-called binary vector technology as disclosed in EP A 120 516 and U.S. Patent 4,940,838.
Tomato transformation is preferably done essentially as described by Van Roekel et al. (Plant Cell Rep. 12, 644-647, 1993). Potato transformation is preferably done essentially as described by Hoekema et al. (Hoekema, A. *et al.*, Bio/Technology 7, 273-278, 1989). Generally, after transformation plant cells or cell groupings are selected for the presence of one or more markers which are encoded by plant expressible genes co-transferred with the nucleic acid sequence encoding the protein according to the invention, whereafter the transformed material is regenerated into a whole plant.

Although considered somewhat more recalcitrant towards genetic transformation, monocotyledonous plants are amenable to transformation and fertile transgenic plants can be regenerated from transformed cells or embryos, or other plant material. Presently, preferred methods for transformation of monocots are microprojectile bombardment of embryos, explants or suspension cells, and direct DNA uptake or electroporation (Shimamoto, *et al*, Nature 338, 274-276, 1989). Transgenic maize plants have been obtained by introducing the *Streptomyces hygroscopicus bar*-gene, which encodes phosphinothricin acetyltransferase (an enzyme which inactivates the herbicide phosphinothricin), into embryogenic cells of a maize suspension culture by microprojectile bombardment (Gordon-Kamm,, Plant Cell, 2, 603-618, 1990). The introduction of genetic material into aleurone protoplasts of other monocot crops such as wheat and barley has been reported (Lee, Plant Mol. Biol. 13, 21-30, 1989). Wheat plants have been regenerated from embryogenic suspension culture by selecting only the aged compact and nodular embryogenic callus tissues for the establishment of the embryogenic suspension cultures (Vasil, Bio/Technol. 8, 429-434, 1990). The combination with transformation systems for these crops enables the application of the present invention to monocots.

Monocotyledonous plants, including commercially important crops such as rice and corn are also amenable to DNA transfer by *Agrobacterium* strains (*vide* WO 94/00977; EP 0 159 418 B1; Gould J, *et al.*, Plant. Physiol. 95, 426-434, 1991).

Following DNA transfer and regeneration, putatively transformed plants may be evaluated, for instance using Southern analysis, for the presence of the chimeric DNA according to the invention, copy number and/or genomic organization. In addition, or alternatively, expression levels of the newly introduced DNA may be undertaken, using Northern and/or Western analysis, techniques well known to persons having ordinary skill in the art. After the initial analysis, which is optional, transformed plants showing the desired copy number and expression level of the newly introduced chimeric DNA according to the invention may be tested for resistance levels against pathogens.. Alternatively, the selected plants may be subjected to another round of transformation, for instance to introduce further genes, in order to enhance resistance levels, or broaden the resistance.

Other evaluations may include the testing of pathogen resistance under field conditions, checking fertility, yield, and other characteristics. Such testing is now routinely performed by persons having ordinary skill in the art.

Following such evaluations, the transformed plants may be grown directly, but usually they may be used as parental lines in the breeding of new varieties or in the creation of hybrids and the like.

To obtain transgenic plants capable of constitutively expressing more than one chimeric gene, a number of alternatives are available including the following:

A. The use of DNA, *e.g* a T-DNA on a binary plasmid, with a number of modified genes physically coupled to a selectable marker gene. The advantage of this method is that the chimeric genes are physically coupled and therefore migrate as a single Mendelian locus.

B. Cross-pollination of transgenic plants each already capable of expressing one or more chimeric genes, preferably coupled to a selectable marker gene, with pollen from a transgenic plant which contains one or more chimeric genes coupled to another selectable marker. Afterwards the seed, which is obtained by this crossing, maybe selected on the basis of the presence of the two selectable markers, or on the basis of the presence of the chimeric genes themselves. The plants obtained from the selected seeds can afterwards be used for further crossing. In principle the chimeric genes are not on a single locus and the genes may therefore segregate as independent loci.

C. The use of a number of a plurality chimeric DNA molecules, *e.g.* plasmids, each having one or more chimeric genes and a selectable marker. If the frequency of co-transformation is high, then selection on the basis of only one marker is sufficient. In other cases, the selection on the basis of more than one marker is preferred.

D. Consecutive transformation of transgenic plants already containing a first, second, (etc), chimeric gene with new chimeric DNA, optionally comprising a selectable marker gene. As in method B,the chimeric genes are in principle not on a single locus and the chimeric genes may therefore segregate as independent loci.

E. Combinations of the above mentioned strategies.

The actual strategy may depend on several considerations as maybe easily determined such as the purpose of the parental lines (direct growing, use in a breeding programme, use to produce hybrids) but is not critical with respect to the described invention.

In this context it should be emphasised that plants already containing chimeric DNA may form a suitable genetic background for introducing further chimeric DNAs according to the invention, for instance in order to enhance the production antipathogenic substances, thereby enhancing resistance levels. The cloning of other genes corresponding to proteins that can suitably be used in combination with the regulatory DNA fragments, and the obtention of transgenic plants, capable of relatively over-expressing same, as well as the assessment of their effect on pathogen resistance *in planta*, is now within the scope of the ordinary skilled person in the art.

Plants with improved resistance against pathogens may be grown in the field, in the greenhouse, or at home or elsewhere. Plants or edible parts thereof may be used for animal feed or human consumption, or may be processed for food, feed or other purposes in any form of agriculture or industry. Agriculture shall mean to include horticulture, arboriculture, flower culture, and the like. Industries which may benefit from plant material according to the invention include but are not limited to the pharmaceutical industry, the paper and pulp manufacturing industry, sugar manufacturing industry, feed and food industry, enzyme manufacturers and the like.
The advantages of the plants, or parts thereof, according to the invention are the decreased need for biocide treatment, thus lowering

costs of material, labour, and environmental pollution, or prolonging shelf-life of products (e.g. fruit, seed, and the like) of such plants. Plants for the purpose of this invention shall mean multicellular organisms capable of photosynthesis, and subject to some form of pathogen attack. They shall at least include angiosperms as well as gymnosperms, monocotyledonous as well as dicotyledonous plants.

EXAMPLE 1

Induction of *ms*59 messengers in sunflower plants.

Leaves of 7 to 8 weeks old Sunflower plants (*Helianthus annuus* cv Zebulon) were induced by spraying 5 times with 5 mM salicylic acid (SA), spraying once with 1mM salicylic acid, once with 0.1 mM jasmonic acid (JA), once with 1 mM ACC (1-aminocyclopropane-1-carboxylic acid, a precursor of the plant hormone ethylene) or wounding. Leaf samples were harvested from induced leaves after 24 hours (1 mM SA, 0.1 mM JA, 1 mM ACC and wounding) and after 5 days (5 mM SA). Control samples were taken at 24 hours after induction in non-induced plants.

EXAMPLE 2

RNA extraction from sunfower leaf tissue and cDNA synthesis.

Total RNA was extracted from 10 g leaf material using a hot phenol method and purified using the Qiagen RNA buffer set and tip-100 columns (Qiagen GmbH, Germany). Contaminating DNA was degraded using a Dnase I (Gibco BRL) treatment.

cDNA was prepared using 1 μg total RNA, 1 μl oligo(dT)$_{12-18}$ primers (500 μg/ml, Gibco BRL) and 200 units of Superscript II RT RNAse H^{-} reverse transcriptase (Gibco BRL) as described by the manufacturer.

EXAMPLE 3

Construction of *ms*59 PCR MIMIC and analysis of samples by competitive RT-PCR.

Transcript levels of *ms*59 were determined using the competitive RT-PCR technology. In this technique competition between the cDNA target and an artificial PCR MIMIC makes quantitation of transcript levels

possible (Paul D. Siebert and James W. Larrick (1992), Nature 359, 557-558).

For construction of a PCR MIMIC the following primers were developed; FR-pUC-208 (SEQ ID NO: 1) 5' GTT CCG GAG GTT GTG ACC GTG GGA TGT GCT GCA AGG CG3', FR-pUC-209 (SEQ ID NO: 2) 5' CTG GGG AAG CCC GTG TAG TAA AGC CCC CGC GCG TTG GCC GAT TC3', FR-MS59-47 (SEQ ID NO: 3) 5' CTG GGG AAG CCC GTG TAG TAA AGC3' and FR-MS59-77 (SEQ ID NO: 4) 5' GTT CCG GAG GTT GTG ACC GTG3'. Primers FR-pUC-208 and FR-pUC-209 were used to amplify a fragment of 387 bp from the plasmid pUC18 (Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) Gene 33, 103-119) by PCR (10 cycles of 1' 95°C, 1' 55°C, 2' 72°C). From this PCR product 1 μl was amplified using primers FR-MS59-47 and FR-MS59-77 by PCR to produce a large amount of PCR MIMIC (30 cycles of 1' 95°C, 1' 55°C, 2' 72°C).

Primers FR-MS59-47 and FR-MS59-77 will amplify a band of 312 bp from the *ms*59 cDNA so it can be distinguished easily from the 387 bp MIMIC when separated on a 2% agarose gel. PCR MIMIC dilutions were made in a range of 100 ng/μl to 0.01 ag/μl in H_2O containing 0.2 μg/μl glycogen as a carrier.

Table 1: Induction levels of the *ms*59 messenger in sunflower leaves after different stress treatments relative to the control.

Induction method	fold induction
Control	1[a]
5 mM salicylic acid	1000
1 mM salicylic acid	1[a]
1 mM ACC	10
0.1 mM jasmonic acid	10
wounding	10

note: [a]: could not be detected, arbitrary set to 1.

The cDNA samples were analysed in a competitive RT-PCR. Therefore 2 μl of the samples was combined in a 0.5 ml tube with 1 μl diluted PCR MIMIC (amounts; 0.1 pg, 10 fg, 1.0 fg, 0.1 fg, 10 ag and 1.0 ag).

Amplification of cDNA and MIMIC was performed using 10 μM of the primers FR-MS59-47 and FR-MS59-77, 0.5 μl 20 mM dNTP's, 1x PCR buffer, $MgCl_2$ and 2.5 units recombinant Taq DNA polymerase (Gibco BRL) and was allowed to proceed for 35 cycles, 1' 95°C, 1' 55°C, 2' 72°C. PCR products were separated on a 2% agarose gel and visualized by staining with ethidium bromide and a UV illuminator.

EXAMPLE 4

Infection assays in sunflower plants wih different fungi.

Fungal infections were performed on plants of 7 to 8 weeks old. Leaves were inoculated by laying small droplets (15-20 μl) of a *Botrytis cinerea* spore suspension, *Diaporthe helianthi* (PH9905) hyphal fragment supension or a *Sclerotinia sclerotiorum* hyphal fragment supsension on small cuttings made in the leaf to enable the fungi to penetrate the plant. Fungal infections were allowed to proceed at 18°C and a high relative humidity (± 90%). Leaf disks (diameter = 13 mm) harbouring the site of infection were harvested at approximately 4 days after inoculation. Around the hole of the first small leaf disk, a further ring of 25 mm was harvested. Leaf disks were also harvested in non-infected leaves around leaf cuttings as a control.

EXAMPLE 5

PolyA$^+$-RNA extraction from sunfower leaf tissue and cDNA synthesis.

Poly-A$^+$ RNA was harvested from 100 mg of leaf tissue using the Quickprep Micro mRNA purification Kit (Amersham Pharmacia Biotech, Uppsala, Sweden). The relative amount of mRNA was determined using visualisation of nucleic acids by spotting 10 μl of the samples with 4 μl 1 μg/ml ethidium bromide on a UV illuminator.

Equal amounts of Poly-A$^+$ RNA (± 100 ng) were used to synthesize cDNA using 200 units of Superscript II RT RNAse H$^-$ reverse transcriptase (Gibco BRL) and 1 μl oligo$(dT)_{12-18}$ primers (500 μg/ml, Gibco BRL) as described by the manufacturer.

Example 6

Analysis of samples by competitive RT-PCR.

The different cDNA samples were analysed as described in example 3.

Table 2: Induction levels of the *ms59* messenger after infection of sunflower leaves with different fungi.

Sample	fold induction leaf disk[b]	fold induction outer infection area[c]
Control	1[a]	1[a]
Diaporthe helianthi	1000	1000
Botrytis cinerea	1000	1[a]
Sclerotinia sclerotiorum	1000	1[a]

note: [a]: arbitrary set to 1.

[b]: leaf disk of 13 mm around site of fungal infection.

[c]: leaf ring from 13 mm to 25 mm around site of fungal infection.

EXAMPLE 7

Construction of a sunflower *gapC* PCR MIMIC and analysis of samples by competitive RT-PCR.

As an internal control on the quality of the mRNA and preparation of cDNA we included a competitive RT-PCR on the housekeeping gene *GapC* using the same samples as described in examples 4,5 and 6.

For the construction of a PCR MIMIC the following primers were developed; FR-pUC-224 (SEQ ID NO: 5) 5' CCA TGG GCT CAA ACT GGA GCC GGC CGG GAG CAG ACA AGC CCG 3', FR-pUC-225 (SEQ ID NO: 6) 5' CGA GAC GTC AAC AGT CGG GAC CCA CTC ATT AGG CAC CCC AGG C3', FR-gapC-211 (SEQ ID NO: 7) 5' CCA TGG GCT CAA ACT GGA GCC G3' and FR-gapC-212 (SEQ ID NO: 8) 5' CGA GAC GTC AAC AGT CGG GAC C3'. Primers FR-pUC-224 and FR-pUC-225 were used to amplify a fragment of 527 bp from the plasmid

pUC18 (Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) Gene 33, 103-119) by PCR (10 cycles of 1' 95°C, 1' 55°C, 2' 72°C). From this PCR product 1 μl was amplified using primers FR-gapC-211 and FR-gapC-212 by PCR to produce a large amount of PCR MIMIC (30 cycles of 1' 95°C, 1' 55°C, 2' 72°C).
Primers FR-gapC-211 and FR-gapC-212 will amplify a band of 470 bp from the *gap*C cDNA so it can be distinguished easily from the 527 bp MIMIC when separated on a 2% agarose gel. PCR MIMIC dilutions were made in a range of 100 ng/μl to 0.01 ag/μl in H_20 containing 0.2 $\mu g/\mu l$ glycogen as a carrier.

The cDNA samples were analysed in a competitive RT-PCR. Therefore 2 μl of each sample was combined in a 0.5 ml tube with 1 μl diluted PCR MIMIC (amounts; 0.1 pg, 10 fg, 1.0 fg, 0.1 fg, 10 ag and 1.0 ag). Amplification of cDNA and MIMIC was performed using 10 μM of the primers FR-gapC-211 and FR-gapC-212, 0.5 μl 20 mM dNTP's, 1x PCR buffer, $MgCl_2$ and 2.5 units recombinant Taq DNA polymerase (Gibco BRL) and was allowed to proceed for 35 cycles. 1' 95°C, 1' 55°C, 2' 72°C. PCR products were separated on a 2% agarose gel and visualized by staining with ethidium bromide and a UV illuminator.

Table 3: Induction levels of the control *Gap*C messenger after infection of sunflower leaves with different fungi.

Sample	fold induction in 13 mm leaf disks
Control	1[a]
Diaporthe helianthi	10
Botrytis cinerea	10
Sclerotinia sclerotiorum	10

note: [a]: arbitrary set to 1.

The results show that RNA quality and cDNA preparation is not affected by these fungal infections. Induction of *GapC* messengers by plant pathogens and environmental stress factors was described before in Laxalt *et al.*, (1996) Plant Mol. Biol. 30: 961-972.

EXAMPLE 8

Isolation of the *ms*59 promoter from the sunflower genome.

For the isolation of the *ms*59 promoter genomic DNA was isolated from sunflower leaves using a CTAB extraction procedure. About 10 µg of genomic DNA was digested with the restriction enzymes *Bsp*H I, *Eco*R V, *Nla* IV, *Hph* I, *Rsa* I, *Ssp* I and *Hin*d III for 16 hours at 37°C. These restriction sites are all located within the first part of the *ms*59 cDNA. The digestion mixtures were extracted with 1 volume phenol:chloroform:isoamylalcohol (25:24:1, v/v, Gibco BRL) and precipitated with 0.1 volume of 3 M NaAc (pH=5.2) and 2.5 volumes of 96% ethanol. The DNA pellet was washed with 70% ethanol and the pellet was then dissolved in 50 µl distilled water.
25 µl of each sample was separated on a 0.7% agarose gel for 16 hours at 40 volts. The DNA was transferred to a nylon membrane (Hybond-N^+, Amersham Life Science) using southern blotting with 0.4 M NaOH. The blot was hybridized (16 hours, 65°C) using a 320 bp fragment (from the ATG startcodon until the *Bsp*H I site) labeled with ^{32}P-dCTP as a probe. Then the blot was washed with a stringency of 0.2x SSC at 65°C. The results of the southern blot are listed in table 4.

The remaining 25 µl of digestion mixture was ligated in such a way that circularization of the DNA fragments was stimulated. This was done by ligating the DNA in a (large) volume of 300 µl in 1x T4 ligation buffer and 5 weiss units of T4 DNA ligase (Gibco BRL) for 16 hours at 16°C. Again the mixture was extracted with phenol:chloroform:isoamylalcohol and precipitated with ethanol and the DNA pellet was dissolved in 50 µl H_2O.

Primers were designed within the first part of the cDNA (between the ATG startcodon and the first restriction site used (*Bsp*H I) and

directed outwards. Primers FR-MS59-11 (SEQ ID NO: 9) 5' CAG GCA GCT GTG GTT TGT GGC3' and FR-MS59-49 (SEQ ID NO: 10) 5' CGG GAA GTT GCA GAA GAT TGG GTT G3' were used in a PCR reaction on 1 µl of the ligation mixture using the Advantage Klentaq polymerase mix (Clontech laboratories, Inc., Palo Alto, CA) 200 µM dNTP's and 10 µM of each primer. The polymerase mix was activated for 1' at 94°C followed by 35 cycles of amplification for 30" at 94°C, 1' at 55°C and 3' at 68°C. The PCR products were analysed on a 1% agarose gel and no specific band could be detected. Therefore nested PCR was performed as described above but now with nested primers FR-MS59-34 (SEQ ID NO: 11) 5' ACG TAG ATA TCG AAC AAG AAA CCG C3' and FR-MS59-50 (SEQ ID NO: 12) 5' GAG CAA GAG AAG AAG GAG AC3' using 1 µl of the PCR product from the first PCR round. After analysis of the PCR products on a 1% agarose gel very specific single bands were detected. Inverse PCR results are listed in table 4.

Table 4: Results inverse PCR and southern blot band sizes (nd= not determined)

Inverse PCR restriction enzyme	Band sizes southern blot	Band sizes nested PCR
BspH I	> 7 kb	nd
EcoR V	> 7 kb	nd
Nla IV	1.0 kb	0.6 kb
Hph I	1.2 kb	0.8 kb
Rsa I	0.5 kb	0.2 kb
Ssp I	1.3 kb	0.9 kb
Hind III	2.3 kb	1.9 kb

The 1.9 kb *Hind* III iPCR band was isolated from gel and the DNA sequence of the ends was determined using primers FR-MS59-34 and FR-MS59-50 on a automatic DNA sequencer (Applied Biosystems).

Based on the DNA sequence new primers were designed for the amplification of the *ms59* promoter region from the sunflower genome. Primer FR-MS59-226 (SEQ ID NO: 13) 5′ GCA AGC TTT ATA GTT TAC GAT CC3′ is directed downstream, located in the upstream part of the *ms59* promoter region overlapping the *Hind* III restriction site. Primer FR-MS59-227 (SEQ ID NO: 14) 5′ TTG CCA TGG TGC ATG GTT TAG CG3′ can anneal at the most downstream part of the *ms59* promoter/leader overlapping the ATG translational start introducing a *Nco* I restriction site spanning the ATG startcodon. The DNA sequence of the complete promoter fragment from the upstream Hind III to Nco I site (SEQ ID NO: 15, nucleotides 1-1889) was determined using automated DNA sequence analysis (Applied Biosystems).

Using *Pfu* DNA polymerase (Stratagene) and both primers the *ms59* promoter region was amplified from sunflower genomic DNA. The 1.9 kb PCR product was digested with *Hind* III and *Nco* I and ligated in a multicopy cloning vector also digested with *Hind* III and *Nco* I. The promoter was fused to the GUSintron reporter gene (Jefferson *et al.*,(1987) EMBO J 6: 3901-3907) followed by the 3′ untranslated region of the potato proteinase inhibitor II gene (Thornburg *et al.*, 1987, Proc. Natl. Acad. Sci. USA 84, 744-748) which contains sequences needed for polyadenylation (An *et al.*, 1989, Plant Cell 1, 115-122) using restriction sites *Nco* I and *Eco*R I resulting in pMOG1367. The entire chimeric gene flanked by restriction sites *Eco*R I and *Hind* III was then transferred to pMOG800 (for description of this plasmid see for example WO 97/42326) digested with *Eco*R I and *Hind* III.
The resulting binary vector pMOG1368 was introduced in *Agrobacterium tumefaciens* strain EHA105 for transformation of target crops potato and tomato, strain MOG101 for transformation of tobacco and *Arabidopsis thaliana* and strain MOG301 for transformation of *Brassica napus*.

Example 9

Transformation of pMOG1368 to potato cv Kardal.

pMOG 1368 was transformed to potato essentially as described by Hoekema et al. (Hoekema, A. *et al.*, Bio/Technology 7, 273-278, 1989). In short, potatoes (*Solanum tuberosum* cv. Kardal) were transformed with the *Agrobacterium* strain EHA 105 pMOG 1368. The basic culture medium was MS30R3 medium consisting of MS salts (Murashige and Skoog (1962) Physiol. Plant. 14, 473), R3 vitamins (Ooms et al. (1987) Theor. Appl. Genet. 73, 744), 30 g/l sucrose, 0.5 g/l MES with final pH 5.8 (adjusted with KOH) solidified when necessary with 8 g/l Daichin agar. Tubers of *Solanum tuberosum* cv. Kardal were peeled and surface sterilized by burning them in 96% ethanol for 5 seconds. The flames were extinguished in sterile water and cut slices of approximately 2 mm thickness. Disks were cut with a bore from the vascular tissue and incubated for 20 minutes in MS30R3 medium containing 1-5 $x10^8$ bacteria/ml of *Agrobacterium* EHA 105 containing the binary vector. The tuber discs were washed with MS30R3 medium and transferred to solidified postculture medium (PM). PM consisted of M30R3 medium supplemented with 3.5 mg/l zeatin riboside and 0.03 mg/l indole acetic acid (IAA). After two days, discs were transferred to fresh PM medium with 200 mg/l cefotaxim and 100 mg/l vancomycin. Three days later, the tuber discs were transferred to shoot induction medium (SIM) which consisted of PM medium with 250 mg/l carbenicillin and 100 mg/l kanamycin. After 4-8 weeks, shoots emerging from the discs were excised and placed on rooting medium (MS30R3-medium with 100 mg/l cefotaxim, 50 mg/l vancomycin and 50 mg/l kanamycin). The shoots were propagated axenically by meristem cuttings.

Example 10

Testing of promoter function in transgenic potato plants

Transgenic potato plants harbouring the pMOG1368 ms59 promoter-GUS construct were grown in tubes in vitro and assayed for expression of the GUS gene. For this purpose leaf, stem and root samples were taken and stained (results in table 5). GUS expression levels were determined visually, on a scale of 0 to 5, where 0 is no detectable expression and 5 is the highest level of GUS we have observed in

leaves of a transgenic plant, of a rare tobacco 35S-GUS-transgenic (line 96306). Samples from leaves of this plant were included in all experiments for internal reference.

Table 5: Expression of the GUS gene driven by the ms59 promoter in leaves, stems and roots of small in vitro plantlets.

Plant number	Leaf	Stem	Root
1368-1	0	0	0
1368-2	0	0	0
1368-3	0	0	0
1368-4	0	0	0
1368-5	0	0	0
1368-6	0	0	0
1368-7	0	0	0
1368-8	0	0	0
1368-9	0	0	0
1368-10	0	0	0
1368-11	0	0	0
1368-12	0	0	1
1368-13	0	0	0
1368-14	0	0	0
1368-15	0	0	0
1368-16	0	0	0
1368-17	0	0	0
1368-18	0	0	1
1368-19	0	0	0
1368-20	0	0	0
1368-21	0	0	0
1368-22	0	0	0
1368-23	0	0	0
1368-24	0	1	0
1368-25	0	0	0
1368-26	0	0	0
1368-27	0	0	0
1368-28	0	0	0
1368-29	0	0	0
1368-30	0	0	0

A selection of in vitro plantlets of the same age were infected with the potato late blight causing fungus *Phytophthora infestans*. Small droplets of water containing a high concentration of fungal spores were applied on the leaf surface. The infection was left to proceed at room temperature for 96 hours. Leaves which showed disease symptoms were removed from the plantlets and stained for expression of the GUS gene by histochemical GUS analysis (Goddijn *et al.*, The Plant Journal (1993) 4(5): 863-873). Expression was monitored in the lesion resulting from the fungal infection, in the primary zone (the area just around the site of infection) and in the uninfected part of the leaf (background).

Table 6: Expression of the GUS gene driven by the ms59 promoter in leaves of potato *in vitro* plantlets infected by *P. infestans*

Plant number	before infection	lesion	primary zone	background
1368-1	0	0	0	0
1368-3	0	0	0	0
1368-4	0	0	1	0
1368-5	0	0	1	0
1368-6	0	0	0	0
1368-7	0	0	0	0
1368-8	0	0	0	0
1368-9	0	0	1	0

Promoter performance was also tested in the leaves of full grown potato plants before and after infection with *P. infestans*. Before inoculation leaves were detached and stained for expression of GUS. The plants were then sprayed with a spore suspension of $5x10^5$ spores/ml and the infection was allowed to develop for 4 days (96 hours). Again leaves were detached and stained for the expression of GUS. The results are listed in table 7. Expression was monitored in the lesion, primary zone and the background area.

Table 7: Expression of the GUS gene driven by the ms59 promoter in leaves of transgenic potato plants before and after infection with *P. infestans*.

Plant number	before infection	lesion	primary zone	background
1368-1	0	0	0	1
1368-2	0	0	0	1
1368-3	0	0	0	0
1368-4	0	0	0	0
1368-5	0	0	0	0
1368-6	0	0	0	0
1368-7	0	0	0	0
1368-9	0	0	0	0
1368-10	0	0	0	0
1368-11	0	0	0	0
1368-12	2	0	2	2
1368-13	0	0	0	0
1368-14	0	0	0	0
1368-15	0	0	0	0
1368-16	0	0	0	0
1368-17	0	0	0	0
1368-18	0	0	2	0
1368-19	0	0	0	0
1368-20	0	0	0	0
1368-21	0	0	0	0
1368-22	0	0	0	0
1368-23	0	0	0	0
1368-24	0	0	0	0
1368-25	0	0	0	0
1368-26	0	0	3	0

The results showed that the *ms*59 promoter responds to fungal infection. The level of induced expression is rather low and may be below the detection level in some cases. This would explain the low frequency of detectable inducible GUS expression.

Claims:

1. A DNA fragment naturally driving the expression of a plant gene coding for hexose oxidase, capable of promoting pathogen-inducible transcription of an associated DNA sequence when re-introduced into a plant.

2. A DNA fragment according to claim 1, characterised in that it is obtainable from *Helianthus annuus*.

3. A DNA fragment according to claim 1, characterised in that it is obtainable from *Lactuca sativa*.

4. A DNA fragment according to claim 2 characterized in that it is the upstream regulatory region of the gene coding for hexose oxidase, denoted as MS59.

5. A DNA fragment according to claim 3, characterized in that it is the upstream regulatory region of the gene coding for hexose oxidase, denoted as WL64.

6. A DNA fragment according to claim 4, characterized in that it comprises the nucleotide sequence from 1 to 1889 depicted in SEQ ID NO: 15.

7. A portion or variant of a DNA fragment according to any one of claims 4 to 6, capable of promoting pathogen-inducible transcription of an associated DNA sequence when re-introduced into a plant.

8. A chimeric DNA sequence comprising in the direction of transcription a DNA fragment according to any one of claims 1 to 7 and a DNA sequence to be expressed under the transcriptional control thereof and which is not naturally under transcriptional control of said DNA fragment.

9. A chimeric DNA sequence according to claim 8, wherein the DNA sequence to be expressed causes the production of an antipathogenic protein.

antipathogenic protein is selected from the group consisting of chitinase, glucanase, osmotin, magainins, lectins, saccharide oxidase, oxalate oxidase, toxins from *Bacillus thuringiensis*, antifungal proteins isolated from *Mirabilis jalapa*, *Amaranthus*, *Raphanus*, *Brassica*, *Sinapis*, *Arabidopsis*, *Dahlia*, *Cnicus*, *Lathyrus*, *Clitoria*, *Allium* seeds, *Aralia* and *Impatiens* and albumin-type proteins, such as thionine, napin, barley trypsin inhibitor, cereal gliadin and wheat-alpha-amylase.

11. A chimeric DNA sequence according to claim 8, wherein the DNA sequence to be expressed causes the production of a protein that can induce a hypersensitive response, preferably selected from the group consisting of Cf, Bs3 and Pto proteins from tomato, Rpm1 and Rps2 from *Arabidopsis thaliana*, N-protein from tobacco, avr proteins from *Cladosporium fulvum*, harpins from *Erwinia* and elicitor proteins (avrBs3, avrRpm1, avrRpt2) from *Pseudomonas* or *Xanthomonas*.

12. A replicon comprising a chimeric DNA sequence according to any one of claims 8 to 11.

13. A replicon comprising in the direction of transcription a DNA fragment according to any one of claims 1 to 7 and at least one recognition site for a restriction endonuclease for insertion of a DNA sequence to be expressed under the control of said DNA fragment.

14. A microorganism containing a replicon according to any one of claims 12 or 13.

15. A plant cell having incorporated into its genome a chimeric DNA sequence according to any one of claims 8 to 11.

16. A plant essentially consisting of cells according to claim 15.

17. A plant according to claim 16 which is a dicotyledonous plant.

18. A part of a plant selected from seeds, flowers, tubers, roots, leaves, fruits, pollen and wood, obtained from a plant according to claim 16 or 17.

identifying homologues capable of promoting pathogen-induced transcription in a plant.

20. Use of a chimeric DNA sequence according to any one of claims 8 to 11 for transforming plants.

21. Use of a portion or variant according to claim 7 for making hybrid regulatory DNA sequences.

22. Use of a chimeric DNA sequence according to any of claims 9 to 11 for conferring pathogen resistance to a plant.

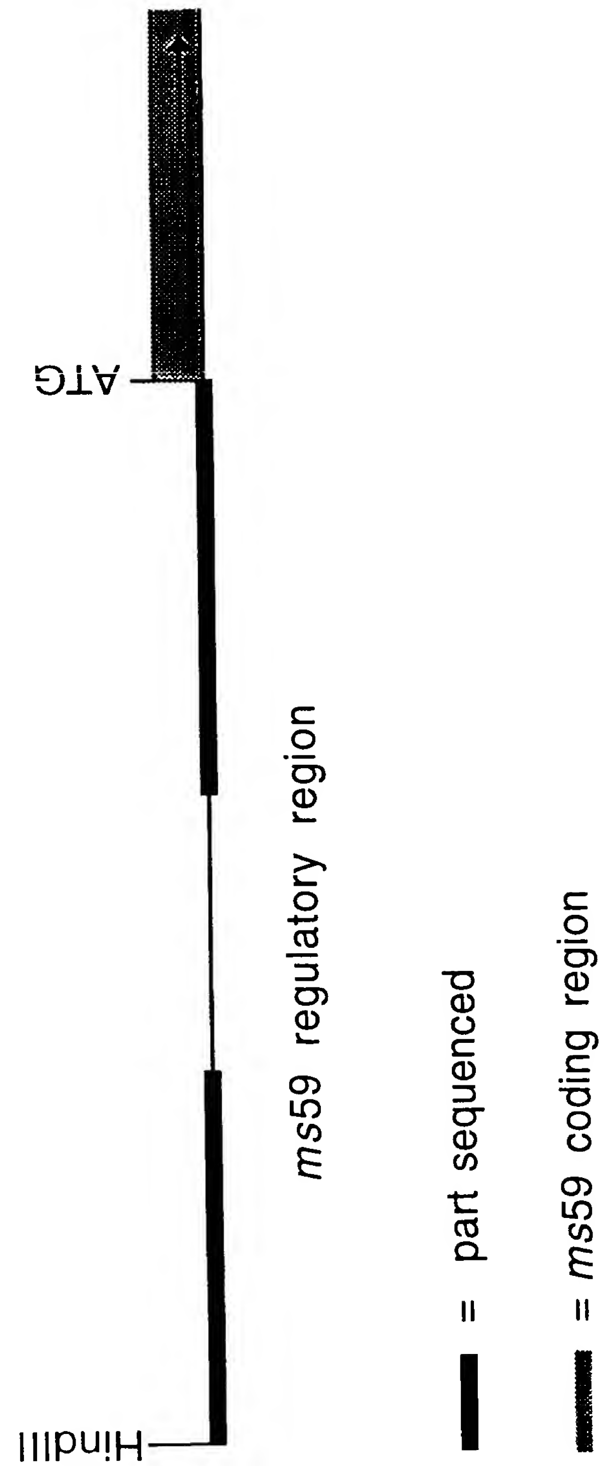
ATG
HindIII
ms59 regulatory region
= part sequenced
= ms59 coding region

Fig. 1

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<210> 14
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 14
ttgccatggt gcatggttta gcg 23

<210> 15
<211> 3680
<212> DNA
<213> Helianthus annuus

<220>
<221> promoter
<222> (1)..(1889)

<220>
<221> CDS
<222> (1890)..(3503)

<400> 15
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aaagtggtgt tccttgaaaa atatttacca aaataatgtt tttgtctcct ttctcatctt 180

aatgaatata aactaaatta ctaattctat tatttatttc tgttaacact ttatttacta 240

ttatttttta gaaacccaat aaaactaaat tagtaatttt attatttatt atattgacac 300

tttattttct ttttgaaact cagacttgag gttctccatt atgtcaccta atattatctt 360

atctaataat ttatacatga ttaacataat ttactcaatt tataattata tctaaattca 420

ctttaacata tattttgtta tttttagttg taacaataaa agtgttaagt tagttagtta 480

taaaaaaggt cagaaagtaa aatagtttag tatgtttgtt aatataagat tacaaaaaag 540

taaaaaaagt aagccataaa aataaaattt gggagttcgt tttctatgac ttgacaacac 600

tttaaagtag tttaatcgat caaaaataca tgatatatta tttatctagt aaaaaataaa 660

aataaaacaa tattagcgta gataagagtg ataaataatt tttttattaa ataattgaaa 720

tttttaaaaa agatcatttt ctaaaaatcc gtagcgagta aagttatgat gtttgtctaa 780

ctttttttatg tttcttattt catactgttt aatatataaa aagataagga gttggtaaaa 840

caaaatataa agagttggtt aaaggtaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaacc 900

tataatggat tgattggagc aaaacaaaat attttattga ttgaataaaa aaatataaca 960

aaaacataat gaataagaag tattacatta cattggcttt aaaaaataat caagtattaa 1020

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tagaaataaa taatgaatta ctaaattagt ttttattcaa tgagataaga atagagaaaa 1080
aaacaccatt ttagtaaata cttttgaaaa acaccatttg atgaatattt tttttcacca 1140
tcactacttt aggaaaaaaa aaaaaaactc ttctttttag tctaatattg agtttttaaa 1200
atacttatca tacttattag acaaaacaat cattttagag taaccttccc ctataccctа 1260
aagtatatat agacgattct aattaaggta ccatataata cagtttaaaa tgatttcatt 1320
aattatccaa aatttttact tttgtttaat tctttttggt tcgtatgtac ttgtataatt 1380
caaagtcttc gtggatttaa attactttga atcaaaaata tgtttgaaat agctgacatg 1440
tgtatttttt caaattgtac ataaatatac gcaaactcga aattgaaaag ttgaaagaaa 1500
acgtgtttca tttttgaaa agtaggcctt atgcaacttg ctatttctc gactatccat 1560
gtttatctcc agatttttaa tttatttgtg tttctttata tctttaacaa gttacaagca 1620
tatggaatta tgtacctatg cactttcact taaaatttat gtcttgttag gagaaattat 1680
tttaaaatca atgttctaat atataaaaat ccaaattaag caatgacact taaaacatat 1740
gaccaatgac acgatatgat gagtacgtac ccaatatgaa ttttcaactt tgattagtta 1800
tatttggtat gtcatttttg aaggtcaaac attacgactt tcaattgcct ataaattgca 1860
tgcatcaagc aaacgctaaa ccatgcaaa atg gca aat atc aca tct tct ttc   1913
                                Met Ala Asn Ile Thr Ser Ser Phe
                                1               5

aac atg caa act tcc att ctt act ctc ctt ctt ctc ttg ctc tca acc   1961
Asn Met Gln Thr Ser Ile Leu Thr Leu Leu Leu Leu Leu Leu Ser Thr
    10                  15                  20

caa tct tct gca act tcc cgt tcc att aca gat cgc ttc att caa tgt   2009
Gln Ser Ser Ala Thr Ser Arg Ser Ile Thr Asp Arg Phe Ile Gln Cys
25                  30                  35                  40

tta cac gac cgg gcc gac cct tca ttt ccg ata acc gga gag gtt tac   2057
Leu His Asp Arg Ala Asp Pro Ser Phe Pro Ile Thr Gly Glu Val Tyr
                45                  50                  55

act ccc gga aac tca tct ttt cct acc gtc ttg caa aac tac atc cga   2105
Thr Pro Gly Asn Ser Ser Phe Pro Thr Val Leu Gln Asn Tyr Ile Arg
            60                  65                  70

aac ctt cgg ttc aat gaa act acc aca cca aaa ccc ttt tta atc atc   2153
Asn Leu Arg Phe Asn Glu Thr Thr Thr Pro Lys Pro Phe Leu Ile Ile
        75                  80                  85

aca gcc gaa cat gtt tcc cac att cag gca gct gtg gtt tgt ggc aaa   2201
Thr Ala Glu His Val Ser His Ile Gln Ala Ala Val Val Cys Gly Lys
    90                  95                  100

caa aac cgg ttg cta ctg aaa acc aga agc ggt ggt cat gat tat gaa   2249
Gln Asn Arg Leu Leu Leu Lys Thr Arg Ser Gly Gly His Asp Tyr Glu
105                 110                 115                 120
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ggt ctt tcc tac ctt aca aac aca aac caa ccc ttc ttc att gtg gac      2297
Gly Leu Ser Tyr Leu Thr Asn Thr Asn Gln Pro Phe Phe Ile Val Asp
                125                 130                 135

atg ttc aat tta agg tcc ata aac gta gat atc gaa caa gaa acc gca      2345
Met Phe Asn Leu Arg Ser Ile Asn Val Asp Ile Glu Gln Glu Thr Ala
            140                 145                 150

tgg gtc caa gcc ggt gcg act ctt ggt gaa gtg tac tat cga ata gcg      2393
Trp Val Gln Ala Gly Ala Thr Leu Gly Glu Val Tyr Tyr Arg Ile Ala
        155                 160                 165

gag aaa agt aac aag cat ggt ttt ccg gca ggg gtt tgt cca acg gtt      2441
Glu Lys Ser Asn Lys His Gly Phe Pro Ala Gly Val Cys Pro Thr Val
    170                 175                 180

ggc gtt ggt ggg cat ttt agt ggt ggt ggg tat ggt aat ttg atg aga      2489
Gly Val Gly Gly His Phe Ser Gly Gly Gly Tyr Gly Asn Leu Met Arg
185                 190                 195                 200

aaa tat ggt ttg tcg gtt gat aat att gtt gat gct caa ata ata gat      2537
Lys Tyr Gly Leu Ser Val Asp Asn Ile Val Asp Ala Gln Ile Ile Asp
                205                 210                 215

gtg aat ggc aag ctt ttg gat cga aag agt atg ggt gag gat ttg ttt      2585
Val Asn Gly Lys Leu Leu Asp Arg Lys Ser Met Gly Glu Asp Leu Phe
            220                 225                 230

tgg gcg atc acc ggc ggt ggt ggt gtt agt ttt ggt gtg gtt cta gcc      2633
Trp Ala Ile Thr Gly Gly Gly Gly Val Ser Phe Gly Val Val Leu Ala
        235                 240                 245

tac aaa atc aaa cta gtt cgt gtt ccg gag gtt gtg acc gtg ttt acc      2681
Tyr Lys Ile Lys Leu Val Arg Val Pro Glu Val Val Thr Val Phe Thr
    250                 255                 260

att gaa aga aga gag gaa caa aac ctc agc acc atc gcg gaa cga tgg      2729
Ile Glu Arg Arg Glu Glu Gln Asn Leu Ser Thr Ile Ala Glu Arg Trp
265                 270                 275                 280

gta caa gtt gct gat aag cta gat aga gat ctt ttc ctt cga atg acc      2777
Val Gln Val Ala Asp Lys Leu Asp Arg Asp Leu Phe Leu Arg Met Thr
                285                 290                 295

ttt agt gtc ata aac gat acc aac ggt gga aag aca gtc cgt gct atc      2825
Phe Ser Val Ile Asn Asp Thr Asn Gly Gly Lys Thr Val Arg Ala Ile
            300                 305                 310

ttt cca acg ttg tac ctt gga aac tcg agg aat ctt gtt aca ctt ttg      2873
Phe Pro Thr Leu Tyr Leu Gly Asn Ser Arg Asn Leu Val Thr Leu Leu
        315                 320                 325

aat aaa gat ttc ccc gag tta ggg ttg caa gaa tcg gat tgt act gaa      2921
Asn Lys Asp Phe Pro Glu Leu Gly Leu Gln Glu Ser Asp Cys Thr Glu
    330                 335                 340

atg agt tgg gtt gag tct gtg ctt tac tac acg ggc ttc ccc agt ggt      2969
Met Ser Trp Val Glu Ser Val Leu Tyr Tyr Thr Gly Phe Pro Ser Gly
345                 350                 355                 360
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act cca acc acg gcg ctc tta agc cgt act cct caa aga ctc aac cca    3017
Thr Pro Thr Thr Ala Leu Leu Ser Arg Thr Pro Gln Arg Leu Asn Pro
                365                 370                 375

ttc aag atc aaa tcc gat tat gtg caa aat cct att tct aaa cga cag    3065
Phe Lys Ile Lys Ser Asp Tyr Val Gln Asn Pro Ile Ser Lys Arg Gln
            380                 385                 390

ttc gag ttc atc ttc gaa agg ctg aaa gaa ctt gaa aac caa atg ttg    3113
Phe Glu Phe Ile Phe Glu Arg Leu Lys Glu Leu Glu Asn Gln Met Leu
        395                 400                 405

gct ttc aac cca tat ggt ggt aga atg agt gaa ata tcc gaa ttc gca    3161
Ala Phe Asn Pro Tyr Gly Gly Arg Met Ser Glu Ile Ser Glu Phe Ala
    410                 415                 420

aag cct ttc cca cat aga tcg ggt aac ata gcg aaa att caa tac gaa    3209
Lys Pro Phe Pro His Arg Ser Gly Asn Ile Ala Lys Ile Gln Tyr Glu
425                 430                 435                 440

gta aac tgg gag gat ctt agc gat gaa gcc gaa aat cgt tac ttg aat    3257
Val Asn Trp Glu Asp Leu Ser Asp Glu Ala Glu Asn Arg Tyr Leu Asn
                445                 450                 455

ttc aca agg ctg atg tat gat tac atg acc cca ttt gtg tcg aaa aac    3305
Phe Thr Arg Leu Met Tyr Asp Tyr Met Thr Pro Phe Val Ser Lys Asn
            460                 465                 470

cct aga aaa gca ttt ttg aac tat agg gat ttg gat att ggt atc aac    3353
Pro Arg Lys Ala Phe Leu Asn Tyr Arg Asp Leu Asp Ile Gly Ile Asn
        475                 480                 485

agc cat ggc agg aat gct tat act gaa gga atg gtt tat ggg cac aag    3401
Ser His Gly Arg Asn Ala Tyr Thr Glu Gly Met Val Tyr Gly His Lys
    490                 495                 500

tat ttc aaa gag aca aat tac aag agg cta gta agt gtg aag act aaa    3449
Tyr Phe Lys Glu Thr Asn Tyr Lys Arg Leu Val Ser Val Lys Thr Lys
505                 510                 515                 520

gtt gat cct gac aac ttc ttt agg aat gag caa agc atc cca act ttg    3497
Val Asp Pro Asp Asn Phe Phe Arg Asn Glu Gln Ser Ile Pro Thr Leu
                525                 530                 535

tca tct tgaagaacgt acatatataa ataaatacct ttgtgcatgg tattttcagg    3553
Ser Ser

gtgttaaagt gatattcaga tatttatgat agaattttga cttgtatttt atacaatcaa 3613

aattgtatgg ttctccgaat ttctcttttt aattctgaaa aatacatatt agtattgtca 3673

aaaaaaa                                                            3680
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<210> 16
<211> 538
<212> PRT
<213> Helianthus annuus

<400> 16

Met Ala Asn Ile Thr Ser Ser Phe Asn Met Gln Thr Ser Ile Leu Thr
1 5 10 15

Leu Leu Leu Leu Leu Leu Ser Thr Gln Ser Ser Ala Thr Ser Arg Ser
20 25 30

Ile Thr Asp Arg Phe Ile Gln Cys Leu His Asp Arg Ala Asp Pro Ser
35 40 45

Phe Pro Ile Thr Gly Glu Val Tyr Thr Pro Gly Asn Ser Ser Phe Pro
50 55 60

Thr Val Leu Gln Asn Tyr Ile Arg Asn Leu Arg Phe Asn Glu Thr Thr
65 70 75 80

Thr Pro Lys Pro Phe Leu Ile Ile Thr Ala Glu His Val Ser His Ile
85 90 95

Gln Ala Ala Val Val Cys Gly Lys Gln Asn Arg Leu Leu Leu Lys Thr
100 105 110

Arg Ser Gly Gly His Asp Tyr Glu Gly Leu Ser Tyr Leu Thr Asn Thr
115 120 125

Asn Gln Pro Phe Phe Ile Val Asp Met Phe Asn Leu Arg Ser Ile Asn
130 135 140

Val Asp Ile Glu Gln Glu Thr Ala Trp Val Gln Ala Gly Ala Thr Leu
145 150 155 160

Gly Glu Val Tyr Tyr Arg Ile Ala Glu Lys Ser Asn Lys His Gly Phe
165 170 175

Pro Ala Gly Val Cys Pro Thr Val Gly Val Gly Gly His Phe Ser Gly
180 185 190

Gly Gly Tyr Gly Asn Leu Met Arg Lys Tyr Gly Leu Ser Val Asp Asn
195 200 205

Ile Val Asp Ala Gln Ile Ile Asp Val Asn Gly Lys Leu Leu Asp Arg
210 215 220

Lys Ser Met Gly Glu Asp Leu Phe Trp Ala Ile Thr Gly Gly Gly Gly
225 230 235 240

Val Ser Phe Gly Val Val Leu Ala Tyr Lys Ile Lys Leu Val Arg Val
245 250 255

Pro Glu Val Val Thr Val Phe Thr Ile Glu Arg Arg Glu Glu Gln Asn
260 265 270

Leu Ser Thr Ile Ala Glu Arg Trp Val Gln Val Ala Asp Lys Leu Asp
275 280 285

Arg Asp Leu Phe Leu Arg Met Thr Phe Ser Val Ile Asn Asp Thr Asn
290 295 300

Gly Gly Lys Thr Val Arg Ala Ile Phe Pro Thr Leu Tyr Leu Gly Asn
305 310 315 320

Ser Arg Asn Leu Val Thr Leu Leu Asn Lys Asp Phe Pro Glu Leu Gly
325 330 335

Leu Gln Glu Ser Asp Cys Thr Glu Met Ser Trp Val Glu Ser Val Leu
340 345 350

Tyr Tyr Thr Gly Phe Pro Ser Gly Thr Pro Thr Thr Ala Leu Leu Ser
355 360 365

Arg Thr Pro Gln Arg Leu Asn Pro Phe Lys Ile Lys Ser Asp Tyr Val
370 375 380

Gln Asn Pro Ile Ser Lys Arg Gln Phe Glu Phe Ile Phe Glu Arg Leu
385 390 395 400

Lys Glu Leu Glu Asn Gln Met Leu Ala Phe Asn Pro Tyr Gly Gly Arg
405 410 415

Met Ser Glu Ile Ser Glu Phe Ala Lys Pro Phe Pro His Arg Ser Gly
420 425 430

Asn Ile Ala Lys Ile Gln Tyr Glu Val Asn Trp Glu Asp Leu Ser Asp
435 440 445

Glu Ala Glu Asn Arg Tyr Leu Asn Phe Thr Arg Leu Met Tyr Asp Tyr
450 455 460

Met Thr Pro Phe Val Ser Lys Asn Pro Arg Lys Ala Phe Leu Asn Tyr
465 470 475 480

Arg Asp Leu Asp Ile Gly Ile Asn Ser His Gly Arg Asn Ala Tyr Thr
485 490 495

Glu Gly Met Val Tyr Gly His Lys Tyr Phe Lys Glu Thr Asn Tyr Lys
500 505 510

Arg Leu Val Ser Val Lys Thr Lys Val Asp Pro Asp Asn Phe Phe Arg
515 520 525

Asn Glu Gln Ser Ile Pro Thr Leu Ser Ser
530 535 538

<210> 17
<211> 1981
<212> DNA
<213> Lactuca sativa

<220>
<221> CDS
<222> (7)..(1626)

<220>
<221> unsure
<222> (372)
<223> replace (372, "g")

<220>
<221> unsure
<222> (379)
<223> replace (379, "g")

<220>
<221> unsure
<222> (786)
<223> replace (786, "t")

<220>
<221> unsure
<222> (1105)..(1106)
<223> replace (1105..1106, "ga" or "gg" or "aa")

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acaaaa atg gca att acc tat tct ttc aac ttc aaa tct tat att ttt 48
Met Ala Ile Thr Tyr Ser Phe Asn Phe Lys Ser Tyr Ile Phe
1 5 10

cct ctc ctc ctt gtc ttg ctc tct acc cat tca tca gcg act tca act 96
Pro Leu Leu Leu Val Leu Leu Ser Thr His Ser Ser Ala Thr Ser Thr
15 20 25 30

tcc att ata gat cgc ttc acc caa tgt cta aac aac cga gct gac cct 144
Ser Ile Ile Asp Arg Phe Thr Gln Cys Leu Asn Asn Arg Ala Asp Pro
35 40 45

tct ttc ccg ctc agt gga caa ctt tac act ccc gat aac tcc tct ttt 192
Ser Phe Pro Leu Ser Gly Gln Leu Tyr Thr Pro Asp Asn Ser Ser Phe
50 55 60

cca tcc gtc ttg caa gct tac atc cgg aac ctc cga ttc aat gaa tcc 240
Pro Ser Val Leu Gln Ala Tyr Ile Arg Asn Leu Arg Phe Asn Glu Ser
65 70 75

acg act ccc aaa ccc atc tta atc atc acc gcc tta cac cct tca cac 288
Thr Thr Pro Lys Pro Ile Leu Ile Ile Thr Ala Leu His Pro Ser His
80 85 90

att caa gca gct gtt gtg tgc gcc aaa aca cac cgc ctg cta atg aaa 336
Ile Gln Ala Ala Val Val Cys Ala Lys Thr His Arg Leu Leu Met Lys
95 100 105 110

acc aga agc gga ggc cat gat tat gag ggg ctt tcc tat gtg acc aat 384
Thr Arg Ser Gly Gly His Asp Tyr Glu Gly Leu Ser Tyr Val Thr Asn
115 120 125

tcg aac caa ccc ttt ttt gtt gtt gac atg ttc aac tta cgc tcc ata 432
Ser Asn Gln Pro Phe Phe Val Val Asp Met Phe Asn Leu Arg Ser Ile
130 135 140

aac gtg agt att gaa gat gaa act gca tgg gtc caa gct ggt gcg act 480
Asn Val Ser Ile Glu Asp Glu Thr Ala Trp Val Gln Ala Gly Ala Thr
145 150 155

ctt ggt gaa gtc tac tac cga ata gca gag aaa agc aac agt cat gct 528
Leu Gly Glu Val Tyr Tyr Arg Ile Ala Glu Lys Ser Asn Ser His Ala
160 165 170

ttt ccg gct ggc gtt tgc cct act gtt gga gtt ggt ggc cat ttt agt 576
Phe Pro Ala Gly Val Cys Pro Thr Val Gly Val Gly Gly His Phe Ser
175 180 185 190

ggt ggt ggt tat ggt aac ttg atg gga aaa tac ggc ctt tct gtt gac 624
Gly Gly Gly Tyr Gly Asn Leu Met Gly Lys Tyr Gly Leu Ser Val Asp
195 200 205

aat att gtc gat gct cag tta atc gat gtg aat ggt aaa ctt ctg aat 672
Asn Ile Val Asp Ala Gln Leu Ile Asp Val Asn Gly Lys Leu Leu Asn
210 215 220

cgg aaa tca atg ggt gaa gat ctt ttt tgg gcc atc aca ggt ggt ggt 720
Arg Lys Ser Met Gly Glu Asp Leu Phe Trp Ala Ile Thr Gly Gly Gly
225 230 235

ggt gtc agc ttt ggt gtg gtt gta gcg tac aag atc aaa ctg gtt cgt 768
Gly Val Ser Phe Gly Val Val Val Ala Tyr Lys Ile Lys Leu Val Arg
240 245 250

gtt cct acc act gtg acc gtt ttt aac gta caa aga aca tcc gag cag 816
Val Pro Thr Thr Val Thr Val Phe Asn Val Gln Arg Thr Ser Glu Gln
255 260 265 270

aac cta agc acc ata gcc cac cga tgg ata caa gtt gcg gat aag ctc 864
Asn Leu Ser Thr Ile Ala His Arg Trp Ile Gln Val Ala Asp Lys Leu
275 280 285

gat aat gac ctt ttc ctt cga atg acc ttt aac gtg ata aac aac aca 912
Asp Asn Asp Leu Phe Leu Arg Met Thr Phe Asn Val Ile Asn Asn Thr
290 295 300

aat ggc gaa aag acg ata cgt ggt ttg ttt cca aca ctg tac ctc gga 960
Asn Gly Glu Lys Thr Ile Arg Gly Leu Phe Pro Thr Leu Tyr Leu Gly
305 310 315

aac tct acc gct ctt gtt gcc ctc ctg aac aag gat ttc cct gaa tta 1008
Asn Ser Thr Ala Leu Val Ala Leu Leu Asn Lys Asp Phe Pro Glu Leu
320 325 330

ggt gta gaa att tca gat tgt att gaa atg agt tgg atc gag tct gtt 1056
Gly Val Glu Ile Ser Asp Cys Ile Glu Met Ser Trp Ile Glu Ser Val
335 340 345 350

ctt ttc tac aca aac ttc ccc att ggt act ccg acc act gct ctt cta 1104
Leu Phe Tyr Thr Asn Phe Pro Ile Gly Thr Pro Thr Thr Ala Leu Leu
355 360 365

agc cgt aca cct caa aga cta aac cca ttc aaa atc aaa tct gat tac 1152
Ser Arg Thr Pro Gln Arg Leu Asn Pro Phe Lys Ile Lys Ser Asp Tyr
370 375 380

gta aaa aac act att tcc aaa cag gga ttc gaa tcc ata ttt gaa agg 1200
Val Lys Asn Thr Ile Ser Lys Gln Gly Phe Glu Ser Ile Phe Glu Arg
385 390 395

atg aaa gaa ctc gaa aac caa atg cta gct ttc aac cct tat ggt gga 1248
Met Lys Glu Leu Glu Asn Gln Met Leu Ala Phe Asn Pro Tyr Gly Gly
400 405 410

aga atg agc gaa att tcc gaa ttt gca aag cct ttt ccc cat cga tca 1296
Arg Met Ser Glu Ile Ser Glu Phe Ala Lys Pro Phe Pro His Arg Ser
415 420 425 430

ggg aat ata gcg aag atc caa tac gaa gta aac tgg gat gaa ctt ggc 1344
Gly Asn Ile Ala Lys Ile Gln Tyr Glu Val Asn Trp Asp Glu Leu Gly
435 440 445

gtt gaa gca gcc aat cgg tac ttg aac ttc aca agg gtg atg tat gat 1392
Val Glu Ala Ala Asn Arg Tyr Leu Asn Phe Thr Arg Val Met Tyr Asp
450 455 460

tat atg act ccg ttt gtt tct aag aac ccc agg gaa gca ttt ctg aac 1440
Tyr Met Thr Pro Phe Val Ser Lys Asn Pro Arg Glu Ala Phe Leu Asn
465 470 475

tac agg gat tta gat att ggt gtc aac agt cat ggc aag aat gct tac 1488
Tyr Arg Asp Leu Asp Ile Gly Val Asn Ser His Gly Lys Asn Ala Tyr
480 485 490

ggt gaa gga atg gtt tat ggg cac aag tat ttc aaa gag acg aat tat 1536
Gly Glu Gly Met Val Tyr Gly His Lys Tyr Phe Lys Glu Thr Asn Tyr
495 500 505 510

aag agg cta acg atg gtg aag acg agg gtt gat cct agc aat ttt ttt 1584
Lys Arg Leu Thr Met Val Lys Thr Arg Val Asp Pro Ser Asn Phe Phe
515 520 525

agg aat gag caa agt atc cca act ttg tca tct tca tgg aag 1626
Arg Asn Glu Gln Ser Ile Pro Thr Leu Ser Ser Ser Trp Lys
530 535 540

taaattctaa attcacttgt gaaattgaat aaaagtatgg ctttttcaag gtcatggtat 1686

ccagattcag atgatattga tataattttg acttgtattt atacaaacaa aattatatta 1746

tatttttctg aatttagatt ttccattctt tggaaaaata tacgaacatt gatgttgata 1806

tttttaagaa ttatagattt tgaacattgt gaacaatgaa taaaccgagg acttcccttg 1866

ggtttttttt ataagtatgt aatagcatgt ctttaatcaa gataaccgat cattggatgc 1926

aatttattat tataaacctt atttaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaa 1981

<210> 18
<211> 540
<212> PRT
<213> Lactuca sativa

<400> 18
Met Ala Ile Thr Tyr Ser Phe Asn Phe Lys Ser Tyr Ile Phe Pro Leu
1 5 10 15

Leu Leu Val Leu Leu Ser Thr His Ser Ser Ala Thr Ser Thr Ser Ile
20 25 30

Ile Asp Arg Phe Thr Gln Cys Leu Asn Asn Arg Ala Asp Pro Ser Phe
35 40 45

Pro Leu Ser Gly Gln Leu Tyr Thr Pro Asp Asn Ser Ser Phe Pro Ser
50 55 60

Val Leu Gln Ala Tyr Ile Arg Asn Leu Arg Phe Asn Glu Ser Thr Thr
65 70 75 80

Pro Lys Pro Ile Leu Ile Ile Thr Ala Leu His Pro Ser His Ile Gln
85 90 95

Ala Ala Val Val Cys Ala Lys Thr His Arg Leu Leu Met Lys Thr Arg
100 105 110

Ser Gly Gly His Asp Tyr Glu Gly Leu Ser Tyr Val Thr Asn Ser Asn
115 120 125

Gln Pro Phe Phe Val Val Asp Met Phe Asn Leu Arg Ser Ile Asn Val
130 135 140

Ser Ile Glu Asp Glu Thr Ala Trp Val Gln Ala Gly Ala Thr Leu Gly
145 150 155 160

Glu Val Tyr Tyr Arg Ile Ala Glu Lys Ser Asn Ser His Ala Phe Pro
165 170 175

Ala Gly Val Cys Pro Thr Val Gly Val Gly Gly His Phe Ser Gly Gly
180 185 190

Gly Tyr Gly Asn Leu Met Gly Lys Tyr Gly Leu Ser Val Asp Asn Ile
195 200 205

Val Asp Ala Gln Leu Ile Asp Val Asn Gly Lys Leu Leu Asn Arg Lys
210 215 220

Ser Met Gly Glu Asp Leu Phe Trp Ala Ile Thr Gly Gly Gly Gly Val
225 230 235 240

Ser Phe Gly Val Val Val Ala Tyr Lys Ile Lys Leu Val Arg Val Pro
245 250 255

Thr Thr Val Thr Val Phe Asn Val Gln Arg Thr Ser Glu Gln Asn Leu
260 265 270

Ser Thr Ile Ala His Arg Trp Ile Gln Val Ala Asp Lys Leu Asp Asn
275 280 285

Asp Leu Phe Leu Arg Met Thr Phe Asn Val Ile Asn Asn Thr Asn Gly
290 295 300

Glu Lys Thr Ile Arg Gly Leu Phe Pro Thr Leu Tyr Leu Gly Asn Ser
305 310 315 320

Thr Ala Leu Val Ala Leu Leu Asn Lys Asp Phe Pro Glu Leu Gly Val
325 330 335

Glu Ile Ser Asp Cys Ile Glu Met Ser Trp Ile Glu Ser Val Leu Phe
340 345 350

Tyr Thr Asn Phe Pro Ile Gly Thr Pro Thr Thr Ala Leu Leu Ser Arg
355 360 365

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Thr Pro Gln Arg Leu Asn Pro Phe Lys Ile Lys Ser Asp Tyr Val Lys
    370                 375                 380

Asn Thr Ile Ser Lys Gln Gly Phe Glu Ser Ile Phe Glu Arg Met Lys
385                 390                 395                 400

Glu Leu Glu Asn Gln Met Leu Ala Phe Asn Pro Tyr Gly Gly Arg Met
                405                 410                 415

Ser Glu Ile Ser Glu Phe Ala Lys Pro Phe Pro His Arg Ser Gly Asn
            420                 425                 430

Ile Ala Lys Ile Gln Tyr Glu Val Asn Trp Asp Glu Leu Gly Val Glu
        435                 440                 445

Ala Ala Asn Arg Tyr Leu Asn Phe Thr Arg Val Met Tyr Asp Tyr Met
    450                 455                 460

Thr Pro Phe Val Ser Lys Asn Pro Arg Glu Ala Phe Leu Asn Tyr Arg
465                 470                 475                 480

Asp Leu Asp Ile Gly Val Asn Ser His Gly Lys Asn Ala Tyr Gly Glu
                485                 490                 495

Gly Met Val Tyr Gly His Lys Tyr Phe Lys Glu Thr Asn Tyr Lys Arg
            500                 505                 510

Leu Thr Met Val Lys Thr Arg Val Asp Pro Ser Asn Phe Phe Arg Asn
        515                 520                 525

Glu Gln Ser Ile Pro Thr Leu Ser Ser Ser Trp Lys
    530                 535                 540
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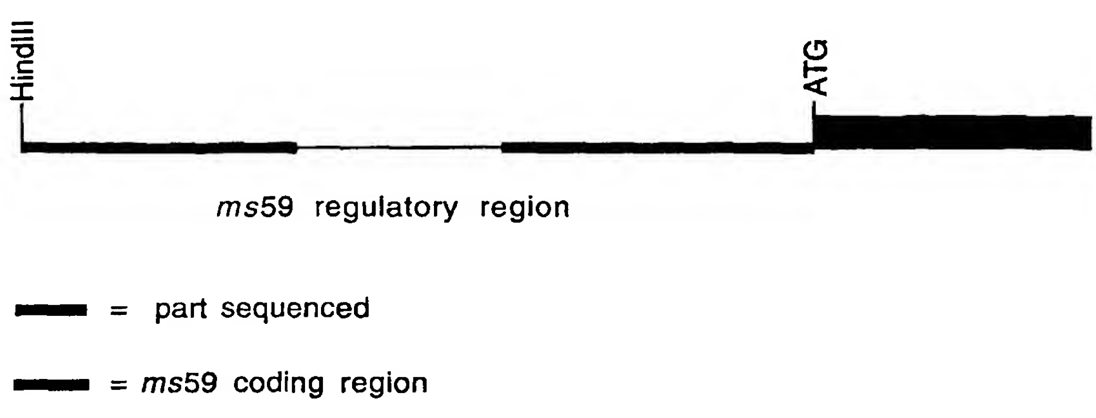
(54) Title: PATHOGEN-INDUCIBLE PROMOTER



(57) Abstract

This invention describes pathogen-inducible promoters which normally drive expression of plant hexose oxidases, especially those which can be isolated from *Helianthus annuus* and *Lactuca sativa*, more specifically those promoters which naturally are the regulatory regions driving expression of the hexose oxidase MS59 and WL64, respectively. Also claimed are chimeric constructs where these pathogen-inducible promoters drive expression of antipathogenic proteins or of proteins which can elicit a hypersensitive response.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/02178

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N5/10 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FACCHINI, P.J., ET AL.: "molecular characterization of berberine bridge enzyme genes from Opium poppy" PLANT PHYSIOLOGY, vol. 112, 1996, pages 1669-1677, XP002111516 the whole document ---	1,7,8, 12-15,20
Y	HAUSCHILD, K., ET AL.: "isolation and analysis of a gene bbel encoding the berberine bridge enzyme from California poppy Eschscholzia californica" PLANT MOLECULAR BIOLOGY, vol. 36, February 1998 (1998-02), pages 473-478, XP002111517 the whole document --- -/--	1,7,8, 12-15,20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
15 October 1999	28.10.99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

7

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/02178

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DITTRICH,H. AND KUTSCHAN, T.M.: "MOLECULAR CLONING, EXPRESSION, AND INDUCTION OF BERBERINE BRIDGE ENZYME, AN ENZYME ESSENTIAL TO THE FORMATION OF BENZOPHENANTHRIDINEALKALOIDS IN THE RESPONSE OF PLANTS TO PATHOGENIC ATTACK" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 88, 1991, pages 9969-9973, XP002025475 the whole document	1,7,8, 12-15,20
A	WO 96 34949 A (COMMW SCIENT IND RES ORG ;UNIV AUSTRALIAN (AU); PRYOR ANTHONY J (A) 7 November 1996 (1996-11-07) abstract, page 6,7,9,20; examples	1-22
A	WO 96 28561 A (MAX PLANCK GESELLSCHAFT ;STRITTMATTER GUENTER (DE)) 19 September 1996 (1996-09-19) pages 1-2,5-7,11; page 15, last pragraph; pages 16,17; examples	1-22
A	EP 0 337 532 A (MOGEN INT ;UNIV LEIDEN (NL)) 18 October 1989 (1989-10-18) column 3, 4, line 26-32; column 6, line 23-42; claims	1-22
A	EP 0 307 841 A (GEN HOSPITAL CORP) 22 March 1989 (1989-03-22) pages 3,5,6,16; claims	1-22
A	WO 98 03536 A (LEBEL EDOUARD GUILLAUME ;RYALS JOHN ANDREW (US); WARD ERIC RUSSELL) 29 January 1998 (1998-01-29) pages 1-5; page 9, line 8-17; claims	1-22
P,X, L	WO 98 13478 A (SELA BUURLAGE MARIANNE BEATRIX ;MELCHERS LEO SJOERD (NL); STUIVER) 2 April 1998 (1998-04-02) page 6, line 37 - page 7, line 5; page 21, line 32 - page 22, line 15; examples;1,4; claims 47-50. ALSO: BBE enzyme is shown to exhibit Hexose Oxidase activity	1-18,20, 22

7

Inte.. ational application No.
PCT/EP 99/02178

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

A meaningful search for the aspect concerning the promoter region of the clone WL64 was not possible due to unsufficient disclosure of the subject matter, i.e. no sequence data were provided.. The search was limited to a keyword search.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

As a result of the prior review under R. 40.2(e) PCT, all additional fees are to be refunded.

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 99/02178

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

A meaningful search for the aspect concerning the promoter region of the clone WL64 was not possible due to unsufficient disclosure of the subject matter, i.e. no sequence data were provided.. The search was limited to a keyword search.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 99/02178

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